Data Evaluation Record

NitroElite/Gamma Aminobutyric Acid (0.5%)

Product Type (EP)

(030802)

Acute Toxicology:

Acute Oral Toxicity (OCSPP 870.1100)
Acute Dermal Toxicity (OCSPP 870.1200)
Acute Inhalation Toxicity (OCSPP 870.1300)
Primary Eye Irritation (OCSPP 870.2400)
Primary Dermal Irritation (OCSPP 870.2500)
Dermal Sensitization (OCSPP 870.2600)

Submitter:	AgXplore International, LLC 7570 State Hwy D Parma, MO 63870
Study Completion Date(s):	04/13/2021
MRID(s):	51605401- 51605406
Primary Review:	Yongqi Li, Biologist, BPPD/RAB
Secondary Review:	Angela Gonzales, Senior Scientist, BPPD/RAB

YONGQI LI Digitally signed by YONGQI LI Date: 2022.04.15 08:48:42 -04'00'

ANGELA GONZALES Digitally signed by ANGELA GONZALES Date: 2022.04.18 09:54:31 -04'00'

STUDY TYPE: Acute Oral Toxicity -Wistar Rat; OCSPP 870.1100

TEST MATERIAL (% a.i): AGX20003D (0.5% GABA), Lot #: 20106.CT.01

SYNONYMS: NitroElite

CITATION: Prajapati J. 2021, Acute Oral Toxicity Study of AGX20003D in Rats. Jai Research Foundation, Valvada – 396 105, Dist. Valsad, Gujarat, India. Laboratory study number 401-1-01-27147, April 2021. Unpublished. MRID No. 51605401.

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EXECUTIVE SUMMARY: In an acute up-and-down oral toxicity study (MRID 51605401), 3 female Wistar rats were given a single oral dose of undiluted test article at a dose of 5000 mg/kg bw. Observations for mortality and clinical/behavioral signs of toxicity were made at 0.5, 1, 2, 3, 4 and 5 hrs on the day of dosing (Day 0) and twice daily thereafter for 14 days. Individual body weights were recorded prior to dosing on day 0, and on Days 7 and 14. There were no deaths and all animals gained weight for the duration of the study. All animals appeared active and healthy during the study. No gross abnormalities were found at necropsy.

Based on the lack of deaths at the limit dose (5000 mg/kg), AGX20003D is classified into EPA Toxicity Category IV for acute oral toxicity.

This acute oral study is classified as acceptable. It does satisfy the guideline requirement for an acute oral toxicity study (OCSPP 870.1100) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was not fully GLP compliant. However, no significant deviations were identified, so the study is considered reliable.

RESULTS and DISCUSSION:

Dose (mg/kg bw)	Mortality/Number Tested		
	Males	Females	Combined
5000	0/0	0/3	0/3

Statistics: No oral LD_{50} was calculated because no mortalities were observed.

A. Mortality – No mortality was observed during the 14 days observation period.

B. Clinical Observations – All the animals were observed for clinical signs of toxicity at 0.5, 1, 2, 3, 4 and 5 hrs post dosing on day 0 and thereafter twice daily for clinical signs of toxicity during the 14 days observation period. No clinical signs of toxicity were observed at dose of 5000 mg/kg. All animals gained weight for the duration of the study. All animals appeared active and healthy during the study.

C. Gross Necropsy – No gross pathological changes were observed.

D. Reviewer's Conclusions: Based on an LD_{50} in rats greater than 5000 mg/kg bw, the test material is classified into EPA Toxicity Category IV for acute oral toxicity. The study was conducted in accordance with the guideline recommendations for an acute oral toxicity study (OCSPP 870.1100) in the rat.

E. Deficiencies: No deficiencies were noted.

STUDY TYPE: Acute Dermal Toxicity -Wistar Rat; OCSPP 870.1200

TEST MATERIAL (% a.i): AGX20003D (0.5% GABA), Lot #: 20106.CT.01

SYNONYMS: NitroElite

CITATION: Prajapati J. 2021, Acute Dermal Toxicity Study of AGX20003D in Rats. Jai Research Foundation, Valvada – 396 105, Dist. Valsad, Gujarat, India. Laboratory study number 403-1-01-27148, April 2021. Unpublished. MRID No. 51605402.

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EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 51605402), five male and five female Wistar rats were dermally exposed to undiluted test article on approximately 10% of the body surface area at dose of 5000 mg/kg bw. Test sites were covered with a semi-occlusive dressing for 24 hours. Animals were observed for 15 days. There were no deaths and all animals gained weight for the duration of the study. All animals appeared active and healthy during the study. No gross abnormalities were found at necropsy.

Based on the lack of deaths at the limit dose (5000 mg/kg), AGX20003D is classified into EPA Toxicity Category IV for acute dermal toxicity.

This acute dermal study is classified as acceptable. It does satisfy the guideline requirement for an acute dermal toxicity study (OCSPP 870.1200) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was not fully GLP compliant. However, no significant deviations were identified, so the study is considered reliable.

RESULTS and DISCUSSION:

Dose (mg/kg bw)	Mortality/Number Tested		
	Males	Females	Combined
5000	0/5	0/5	0/10

Statistics – No dermal LD_{50} was calculated because no mortalities were observed.

A. Mortality – No mortality was observed during the 15 days observation period.

B. Clinical Observations – All the animals were observed for clinical signs of toxicity at 1, 2, 3 and 4 h post dosing on day 0 and thereafter twice daily for clinical signs of toxicity during the 15 days observation period. No clinical signs of toxicity were observed at dose of 5000 mg/kg bw in any of the dosed animals. The body weight was recorded on day 0 before test item application and on day 7 and 14. All animals gained weight for the duration of the study. All animals appeared active and healthy during the study.

C. Gross Necropsy – No gross pathological changes were observed.

D. Reviewer's Conclusions: Based on an LD_{50} in rats greater than 5000 mg/kg bw, the test material is classified into EPA Toxicity Category IV for acute dermal toxicity. The study was conducted in accordance with the guideline recommendations for an acute dermal toxicity study (OCSPP 870.1200) in the rat.

E. Deficiencies: No deficiencies were noted.

STUDY TYPE: Acute Inhalation Toxicity -Wistar Rat; OCSPP 870.1300

TEST MATERIAL (% a.i): AGX20003D (0.5% GABA), Lot #: 20106.CT.01

SYNONYMS: NitroElite

CITATION: Prajapati J. 2021, Acute Inhalation Toxicity Study of AGX20003D in Rats. Jai Research Foundation, Valvada – 396 105, Dist. Valsad, Gujarat, India. Laboratory study number 405-1-01-27149, April 2021. Unpublished. MRID No. 51605403.

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EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 51605403), five male and five female Wistar rats were exposed nose only via the inhalation route to undiluted test article for 4 hours at concentration of 2.138 mg/L with the MMAD and GSD of 3.43 μm and 1.64, respectively. Animals were then observed for 15 days. There were no deaths and all animals gained weight for the duration of the study. All animals appeared active and healthy during the study. No gross abnormalities were found at necropsy.

Based on the lack of deaths at the limit concentration (2.138 mg/L), AGX20003D is classified into EPA Toxicity Category IV for acute inhalation toxicity.

This acute inhalation study is classified as acceptable. It does satisfy the guideline requirement for an acute inhalation toxicity study (OCSPP 870.1300) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was not fully GLP compliant. However, no significant deviations were identified, so the study is considered reliable.

RESULTS and DISCUSSION:

Nominal Conc. (mg/L)	Actual Conc. (Gravimetric/ Analytical) (mg/L)	MMAD µm	GSD	Mortality/Number Tested		
				Males	Females	Combined
8.869	2.138	3.43	1.64	0/5	0/5	0/10

Test Atmosphere / Chamber Description:

Chamber Air Flow (changes/hour)	378-379
Chamber Volume (L):	2.062
Total Airflow (LPM):	13
Temperature (°C):	21.1 – 21.5 ºC
Relative Humidity (%):	60.9 – 62.2%
T_{95} (minutes):	0.48

Test Atmosphere Concentration: Nominal concentration was calculated according to "mass of test item disseminated into the exposure system during the generation period divided by the total airflow through the inhalation chamber during the same time period". It is 8.869 mg/L.

Particle size determination: Particle size distribution was measured gravimetrically using a seven stage Cascade Impactor. Based on the results of mass deposited on every stage, MMAD and GSD were calculated. During sampling, the Impactor air flow rate was 0.74 L/min. The particle size was measured twice during exposure period at animal breathing zone. MMAD is calculated directly from percent particle size distribution. The mean MMAD and GSD were found to be 3.43 µm and 1.64, respectively.

Statistics: No LC_{50} was calculated since no mortalities were observed.

A. Mortality – No mortality was observed during the 15 days observation period.

B. Clinical Observations – All the animals were observed for clinical signs during exposure and post-exposure on day 0 and twice daily thereafter during the 15 days observation period. All animals gained weight for the duration of the study. All animals appeared active and healthy during the study.

C. Gross Necropsy – No gross pathological changes were observed.

D. Reviewer's Conclusions: Based on an LC_{50} in rats greater than 2.138 mg/L, the test material

is classified into EPA Toxicity Category IV for acute inhalation toxicity. The study was conducted in accordance with the guideline recommendations for an acute inhalation toxicity study (OCSPP 870.1300) in the rat.

E. <u>Deficiencies</u>: No deficiencies were noted.

STUDY TYPE: Primary Eye Irritation – NW Rabbit; OCSPP 870.2400

TEST MATERIAL (% a.i): AGX20003D (0.5% GABA), Lot #: 20106.CT.01

SYNONYMS: NitroElite

CITATION: Prajapati J. 2021, Acute Eye Irritation Study of AGX20003D in Rabbits. Jai Research Foundation, Valvada – 396 105, Dist. Valsad, Gujarat, India. Laboratory study number 407-1-01-27151, April 2021. Unpublished. MRID No. 51605405.

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EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 51605405), 0.1 ml of undiluted test article was instilled into the conjunctival sac of the right eyes of three female New Zealand White rabbits for 24 hours. The eyes were washed using normal saline after 24 hours of treatment. Animals were then observed for three days. Irritation was scored according to Draize method of scoring (Draize, Woodard, & Calvery, 1944). There were no observations of iritis, conjunctival chemosis, or corneal opacity at any time during the study. One hour after test substance instillation, all three treated eyes exhibited conjunctival redness (all scores = 1). All eyes were clear of effects at 24 hours. The maximum mean total score (MMTS) was 1.0 at one hour after instillation. No abnormal clinical effects were observed, and all animals gained weight during the study. No gross pathological findings were observed.

In this study, AGX20003D is not irritating or corrosive to the eye based on the minimal effects clearing in less than 24 hours. The test substance is assigned Toxicity Category IV.

This primary eye irritation study is classified as acceptable. It does satisfy the guideline requirement for a primary eye irritation study (OCSPP 870.2400) in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was not fully GLP compliant. However, no significant deviations were identified, so the study is considered reliable.

RESULTS AND DISCUSSION:

Observations	Number "positive"/number tested			
	Hours post instillation			
	1	24	48	72
Corneal Opacity	0/3	0/3	0/3	0/3
Iritis	0/3	0/3	0/3	0/3
Conjunctivae:				
Redness[1]	0/3	0/3	0/3	0/3
Chemosis[1]	0/3	0/3	0/3	0/3
Discharge	0/3	0/3	0/3	0/3
Severity of Irritation: Mean Score	1	0	0	0

[1] Score of 2 or more required to be considered "positive"

A. <u>Observations</u> – The eyes were scored approximately at 1, 24, 48 and 72 hours after exposure to the test material. All the animals were observed twice daily for clinical signs of toxicity or mortality. No clinical signs of toxicity or mortality were observed in all the animals after exposure to the test material. Treated eyes revealed conjunctival redness (score = 1.0) at 1 hour observation. The observed redness was cleared at 24 hours observation. No ocular irritation was observed at or after 24 hours observation. All animals gained weight for the duration of the study. All animals appeared active and healthy during the study. No gross pathological findings were observed.

B. <u>Reviewer's Conclusions</u> – Based on minimal effects clearing in less than 24 hours, the test material is classified in EPA Toxicity Category IV for primary eye irritation. The study was conducted in accordance with the guideline recommendations for a primary eye irritation study (OCSPP 870.2400) in the rabbit.

C. <u>Deficiencies</u>: No deficiencies were noted.

STUDY TYPE: Primary Dermal Irritation – NW Rabbit; OCSPP 870.2500

TEST MATERIAL (% a.i): AGX20003D (0.5% GABA), Lot #: 20106.CT.01

SYNONYMS: NitroElite

CITATION: Prajapati J. 2021, Acute Dermal Irritation Study of AGX20003D in Rabbits. Jai Research Foundation, Valvada – 396 105, Dist. Valsad, Gujarat, India. Laboratory study number 406-1-01-27150, April 2021. Unpublished. MRID No. 51605404.

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EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 51605404), three male New Zealand White rabbits were dermally exposed to 0.5 ml of undiluted test article on the body surface area – left dorsal region. Test sites were covered with a semi-occlusive dressing for 4 hours. After exposure, the test patches were removed, and the test sites were washed with distilled water. Animals were then observed for 3 days. Irritation was scored using the Draize Scale. Very slight erythema was observed in all rabbits at 1, 24, and 48 h post patch removal which resolved by 72 h post patch removal and very slight oedema was observed in all rabbits at 1 and 24 h post patch removal which resolved by 48 h post patch removal. All animals gained weight during the study. All animals appeared normal throughout the study.

In this study, AGX20003D is slightly irritating to the skin based on the primary irritation index (PII) of 1.25. Based on the lack of irritation signs at 72 hours, the test substance is assigned Toxicity Category IV.

This primary dermal irritation study is classified as acceptable. It does satisfy the guideline requirement for a primary dermal irritation study (OCSPP 870.2500) in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was not fully GLP compliant. However, no significant deviations were identified, so the study is considered reliable.

RESULTS and DISCUSSION:

Animal Number	Sex	Skin Irritation Following Patch Removal			
		30-60 minutes*	Hours		
			24*	48*	72*
1	Male	[a] 1/1	1/1	1/0	0/0
2	Male	1/1	1/1	1/0	0/0
3	Male	1/1	1/1	1/0	0/0
Severity of Irritation: Mean Score		2	2	1	0

***** Used in calculation of Primary Irritation Index (PII).

[a] Erythema/Edema

A. <u>Observations</u> – The application sites were observed and scored approximately at 1, 24, 48 and 72 hours after test material removal, all the animals were observed once daily for clinical signs of toxicity and twice daily for mortality during the observation period. very slight erythema was observed in all rabbits at 1, 24, and 48 h post patch removal which resolved by 72 h post patch removal and very slight oedema was observed in all rabbits at 1 and 24 h post patch removal which resolved by 48 h post patch removal. No clinical signs of toxicity and mortality was observed in the test animals. All animals gained weight for the duration of the study. All animals appeared active and healthy during the study.

B. <u>Reviewer's Conclusions</u> – Based on the PII of 1.25, the test material was slightly irritating. There is no irritation at 72 hours, and the test material is classified into EPA Toxicity Category IV. The study was conducted in accordance with the guideline recommendations for a primary dermal irritation study (OCSPP 870.2500) in the rabbit.

C. <u>Deficiencies</u>: No deficiencies were noted.

STUDY TYPE: Dermal Sensitization - Buehler Method in Guinea Pigs; OPPTS 870.2600

TEST MATERIAL (% a.i): AGX20003D (0.5% GABA), Lot #: 20106.CT.01

SYNONYMS: NitroElite

CITATION: Prajapati J. 2021, Skin Sensitization Study of AGX20003D in Guinea Pigs. Jai Research Foundation, Valvada – 396 105, Dist. Valsad, Gujarat, India. Laboratory study number 408-1-01-27152, April 2021. Unpublished. MRID No. 51605406.

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EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 51605406) with undiluted test article, 15 male and 15 female Hartley Guinea Pigs were tested using the Buehler Test Method. Following challenge, there was no indication of a sensitization response in the test or naïve control animals at 24 or 48 hours after exposure. No abnormal clinical signs were observed.

In this study, AGX20003D is not a dermal sensitizer. Positive control substance and positive control study results and methodology were appropriate.

This dermal sensitization study is classified as acceptable. It does satisfy the guideline requirement for a dermal sensitization study (OCSPP 870.2600).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was not fully GLP compliant. However, no significant deviations were identified, so the study is considered reliable.

I. PROCEDURE

A. Irritation Range-Finding – In the pre-study, no skin reactions were observed at the highest dose, i.e., undiluted test material (AGX20003D, 0.5% GABA). Hence, the undiluted test material was selected for induction and challenge phase of the main study.

B. Induction – On days 0, 7 and 14, 0.2 ml undiluted test material was applied topically on the left flank regions of the animals. Approximately 4 cm × 2 cm of cotton gauze was covered on the applied area of the skin and held in place with non-irritating adhesive tape for 6 hours. Then the test patches were removed, and the areas were cleaned with distilled water. The test material application sites were examined and scored for skin reaction at 24 hours after the removal of the test patches.

C. Challenge – On day 28, 0.2 ml undiluted test material was applied topically on the right flank regions of the animals. Approximately 4 cm × 2 cm of cotton gauze was covered on the applied area of the skin and held in place with non-irritating adhesive tape for 6 hours. Then the test patches were removed, and the areas were cleaned with distilled water. The test material application sites were examined and scored at 24 and 48 hours after the removal of the test patches.

D. Positive and Negative Controls – The positive control responses were validated using α-hexylcinnamaldehyde, which was conducted within 6 months of the current study. Distilled water (0.2 mL) served as the negative control.

II. RESULTS and DISCUSSION:

A. Reactions and Duration – No clinical signs of toxicity and mortality were observed in the animals during the study. No erythema or oedema were observed in both the negative control and treatment group animals in induction phase at 24-hours observations after test material patch removal. No skin reactions were observed in both the negative control and treatment group at 24- and 48-hours observations after test material patch removal. All animals gained weight for the duration of the study. All animals appeared active and healthy during the study. No gross pathological findings were observed.

B. Positive Control – α-hexylcinnamaldehyde (97.2%) showed a mean skin sensitization rate of 30% in the challenge phase. The results of the positive control study confirmed the sensitivity of the Buehler Test Method.

C. Reviewer's Conclusions – Under the conditions of the study, AGX20003D is not a dermal sensitizer. The study was conducted in accordance with the guideline recommendations for a dermal sensitization study (OCSPP 870.2600) in the guinea pig.

D. Deficiencies: No deficiencies were noted.